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# Acute Toxic Effects of An Isolate of Moniliformin-Producing *Fusarium oxysporum* and Purified Moniliformin on Rats

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**Abstract.** An isolate of *Fusarium oxysporum* (MT-6) was obtained from pasture soil in New Zealand in 1987. The isolate was grown on rice and fed to rats in a ratio of 50% and 10% of a complete diet. All rats died within 16 hr and showed mild intestinal and thymic hemorrhage. The major identifiable toxin was moniliformin found at a concentration of 9 mg/g in the rice culture. Purified crystals of moniliformin fed to rats at concentrations of 8, 5 and 3 mg/g of rat diet caused hemorrhaging in the intestines and death of 3 of 3 rats within 16 hr. Dietary concentrations of 2.5 and 2 mg/g of moniliformin killed 2 of 3 rats and concentrations of 1.5 and 1 mg/g killed 1 of 3 rats. No lesions were observed in 3 groups of 3 rats fed a diet containing 0.75, 0.5 or 0.25 mg/g of moniliformin. Intragastric intubation of moniliformin caused hemorrhage of the small intestine and death in 5 of 5 rats at each concentration of 100, 80, 60 and 40 mg/kg body weight and death of 4 of 5 rats at 20 mg/kg. No observable toxic effects were observed in groups of 5 rats each receiving 10, 5 and 2.5 mg of moniliformin/kg body weight.

Moniliformin, a mycotoxin and phytotoxic metabolite of *Fusarium* species was discovered by (Cole *et al.* 1973), while screening for toxic products of *F. moniliforme* cultured on corn. Many toxic cultures of *F. oxysporum* isolated from cereal grains are capable of producing moniliformin (Abbas *et al.* 1988b, 1988c; Marasas *et al.* 1979, 1984). Moniliformin has been found in nature on cereal grains together with other toxins such as deoxynivalenol (DON), zearalenone (ZEA) and fusarin C (Theil *et al.* 1982, 1986). Reports have shown that high levels of moniliformin (range, 3.7 to 50 mg/kg) are toxic to chicks, mice, ducklings, rats and sheep (Allen *et al.* 1981; Burmeister *et al.* 1979, 1980; Lamprecht *et al.* 1986; Kriek *et al.* 1977).

The experiments were made to determine whether moniliformin found in cultures of *Fusarium* grown in the laboratory can account for the toxic signs and death of rats in the biological screen designed to detect toxic *Fusarium* isolates. Moreover, it was necessary to establish what dietary concentration is toxic. The toxicity of moniliformin by stomach intubation is much more severe than by introduction through the diet. The present study reports the acute effects of dietary moniliformin.

## Materials and Methods

### *Isolation of* Fusarium oxysporum

The soil-plate method of (Warcup 1950) was used to isolate fungi from pasture soil sample No. MT-6, which was collected in New Zealand (Dunedin) in 1987. *Fusarium oxysporum* MT-6 was identified according to the manual of (Nelson *et al.* 1983). The stock culture of the isolate was maintained in moist autoclaved soil stored at −15°C.

### *Growth of* Fusarium oxysporum

The *F. oxysporum* isolate MT-6 was grown on a rice medium prepared in 1 L flasks (Abbas *et al.* 1984) for 2 weeks at 25°C and then at 12°C for 2 weeks. Each flask was shaken daily for the first few days after inoculation to ensure that the fungus uniformly penetrated the rice. After 4 wk, the rice culture was broken up, transferred to a screen-bottom tray, air-dried in a ventilated hood, and ground to the consistency of flour in a coffee grinder.

### *Rat Feeding Toxicity Study*

The ground culture medium of *F. oxysporum* MT-6 was mixed 50% or 10% with a finely divided complete rat diet, and fed to 20-day virgin female mostly of the Sprague-Dawley rats (Bio-Lab Corp, St. Paul, MN). Female Wistar rats (Sprague-Dawley, P.O. Box 29176, Indianapolis, IN) were used to demonstrate that the culture was toxic to more than one strain of rat. The rats were housed in individual cages and cared for in accordance with the guide for the care and use of laboratory animals (National Institute of Health, Bethesda, MD and University of Minnesota, Minneapolis, MN). The rats were judged to be healthy on the basis of visual examination, level of activity, appetite, and rate of gain.

In a separate set of experiments, the rats received pure moniliformin toxin (purity 99% by HPLC) mixed with complete rat diet at levels of 8, 5, 3, 2.5, 2, 1.5, 1, 0.75, 0.5, and 0.25 mg of monili-

**Table 1.** Dietary toxicity of *Fusarium oxysporum* MT-6 grown on rice in culture and fed to rats

| Sample[a] | Avg. wt. change (g) | Avg. food consumption (g) | Mortality/ number | Toxic signs |
|---|---|---|---|---|
| Control 1 | 2.3 ± 0.5 | 7.1 ± 0.4 | 0/6 | a |
| Control 2 | 2.6 ± 0.7 | 8.0 ± 0.7 | 0/6 | a |
| 50% rice culture[b] | ND | 3.2 ± 1.1 | 27/27 | b,In |
| 50% rice culture[c] | ND | 4.2 ± 1.0 | 6/6 | b,In |
| 10% rice culture[b] | ND | 6.3 ± 1.5 | 3/6 | b,In |
| Residue 1 | ND | 3.5 ± 1.2 | 3/3 | b,In |
| Residue 2 | ND | 4.0 ± 1.0 | 3/3 | b,In |
| Residue 3 | ND | 4.1 ± 0.7 | 3/3 | b,In |
| Residue 4 | 1.2 ± 0.3 | 8.0 ± 1.5 | 0/3 | a |
| Residue 5 | 2.0 ± 0.5 | 7.2 ± 1.2 | 0/3 | a |
| Residue 6 | 1.5 ± 0.4 | 5.7 ± 1.5 | 0/3 | a |

[a] Control 1 = complete rat diet only; Control 2 = 50% autoclaved rice in complete rat diet; Residues = remaining rice culture after extraction with chloroform, residue 1; ethylacetate, residue 2; acetone, residue 3; 95% acetonitrile, residue 4; MeOH, residue 5; and 50% MeOH, residue 6. ND = not determined due to death. a = no toxic signs; b = toxic sign detected; and In = intestinal hemorrhage
[b] Twenty-day-old virgin female Sprague-Dawley rats (Bio-Lab Corp, St. Paul, MN)
[c] Twenty-day-old virgin female Wistar (Sprague Dawley, P.O. Box 29176, Indianapolis, IN)

**Table 2.** Toxicity of solvent extracts of *F. oxysporum* MT-6 to female white rats when administered by oral intubation

| Extracts[a] | Dosage (g infected rice medium extracted per 0.5 ml 20% ethanol) | Mortality/ number | Toxic[b] signs |
|---|---|---|---|
| Control | 1.25 | 0/3 | a |
| | 2.5 | 0/3 | a |
| Solvent carrier (20% ethanol) | 0.5 ml | 0/6 | a |
| Chloroform | 1.25 | 0/3 | a |
| | 2.5 | 0/3 | a |
| Ethylacetate | 1.25 | 0/3 | a |
| | 2.5 | 0/3 | a |
| Acetone | 25 | 0/3 | a |
| Methanol | 1.25 | 6/6 | b,In |
| | 2.5 | 6/6 | b,In |
| 50% MeOH in $H_2O$ | 1.25 | 6/6 | b,In |
| | 2.5 | 6/6 | b,In |
| 95% acetonitrile in $H_2O$ | 1.25 | 6/6 | b,In |
| | 2.5 | 6/6 | b,In |

[a] Control = complete rat diet extracted with 50% MeOH in $H_2O$. Solvent carrier (20% ethanol) used as control (0.5 ml)
[b] a = no toxic signs; b = toxic effects; and In = intestinal hemorrhage

formin/g of complete rat diet. The animals' weight and feed consumption were recorded at the beginning and end of the experiment. Three rats were used for each experimental treatment. Six control rats were fed a 1:1 mixture of autoclaved rice with complete rat diet and 6 other rats were fed a complete rat diet. Rats were observed frequently for 5 days and signs and time of death were recorded. Surviving rats were sacrificed by cervical dislocation and examined for gross pathological changes.

## *Gastric Intubation Test*

Fifty grams of rice culture of *F. oxysporum* MT-6 were extracted twice with either chloroform, ethyl acetate, methanol, 50% methanol in water, or acetonitrile:water (95:5 v/v) for one hour each with 250 ml of solvent. Each extract was intubated into rats at levels of 2.5 g and 1.25 g equivalent of rice culture per 0.5 ml 20% ethanol. A red pigment(s) was extracted from cultured rice with acetone. A 25 g culture equivalent of crude pigment was intubated into rats in 0.5 ml 20% ethanol. Three rats were used per treatment. Moniliformin crystals (99%) dissolved in sterilized distilled water (2.5 ml) were intubated into rats at levels of 100, 80, 60, 40, 20, 10, 5, and 2.5 mg/kg body weight. Five rats were used per treatment and each of 5 control rats were intubated with 0.5 ml of sterile distilled water.

Treated and control rats were observed frequently for 5 days and major signs and time of death recorded. Surviving rats were killed by cervical dislocation and examined for gross pathological changes.

## *Detection of Mycotoxins in* Fusarium *Extracts*

The mycotoxins T-2 toxin, HT-2 toxin, T-2 tetraol, diacetoxyscirpenol (DAS), monoacetoxyscirpenol (MAS), deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxyniva-

**Table 3.** Body weight gain, feed consumption, and mortality of white female (50 g) rats fed moniliformin (pure, crystal form)

| Dietary[a] moniliformin (mg/g of diet) | Body weight gain/loss (g) | Feed consumption (g) | Amount of MON consumed by rat (mg) | Mortality/ number | Toxic[b] signs |
|---|---|---|---|---|---|
| 0 | 1.5 ± 0.7 | 6.3 ± 1.0 | 0 | 0 | a |
| 0.25 | −2.0 ± 1.2 | 5.6 ± 1.3 | 1.4 | 0 | a |
| 0.5 | −2.0 ± 1.0 | 5.0 ± 0.8 | 2.5 | 0 | a |
| 0.75 | −3.2 ± 1.0 | 5.3 ± 1.2 | 4.0 | 0 | a |
| 1.0 | −4.0 ± 1.5 | 5.3 ± 1.2 | 5.3 | 1/3 | a |
| 1.5 | −3.7 ± 0.7 | 3.9 ± 1.6 | 5.9 | 1/3 | a |
| 2.0 | −3.5 ± 0.5 | 3.6 ± 1.2 | 7.2 | 2/3 | b,In |
| 2.5 | −3.0 ± 1.0 | 2.9 ± 1.0 | 7.3 | 2/3 | b,In |
| 3.0 | −1.9 ± 0.8 | 2.9 ± 1.0 | 8.7 | 3/3 | b,In |
| 5.0 | −3.1 ± 1.2 | 2.0 ± 0.5 | 10.0 | 3/3 | b,In |
| 8.0 | −3.0 ± 1.2 | 1.3 ± 0.2 | 10.4 | 3/3 | b,In |

[a] Moniliformin offered as 99% purity crystals (Na salt) mixed with diet and offered to rats for 24 hr
[b] (a) = no toxic effects; (b) = toxicity with (In) intestinal hemorrhage

**Table 4.** Acute toxicity of moniliformin administered by gastric intubation to rats

| Dosage[a] moniliformin (mg/kg body wt) | Absolute amt./rat (mg) | Mortality/ number | Toxic[b] signs |
|---|---|---|---|
| Control ($H_2O$, 0.5 ml) | — | 0/5 | a |
| 2.5 | 0.125 | 0/5 | a |
| 5 | 0.25 | 0/5 | a |
| 10 | 0.5 | 0/5 | a |
| 20 | 1.0 | 4/5 | b,In |
| 40 | 2.0 | 5/5 | b,In |
| 60 | 3.0 | 5/5 | b,In |
| 80 | 4.0 | 5/5 | b,In |
| 100 | 5.0 | 5/5 | b,In |

[a] Five, white, virgin, female, weanling rats (50 g body wt.) were intubated with the sodium form of moniliformin in 0.5 ml water. The experiment was repeated with similar results
[b] (a) = no toxic signs
(b) = toxicity as shown by (In) intestinal hemorrhage

lenol (15-ADON), nivalenol, fusarenon-X, zearalenone, alpha- and beta-zearalenols and fusarochromanone (TDP-1), were analyzed by the method described by Abbas *et al.* (1984). Wortmannin detection was by the method described by (Abbas and Mirocha 1988a). Moniliformin detection, quantitation and confirmation was by methods described by (Scott and Lawrence 1987) and (Vesonder 1986) which utilize thin layer chromatography (TLC) and ultraviolet spectroscopy.

## Results and Discussion

*Fusarium oxysporum*, isolate MT-6, was found in pasture soil. Moniliformin (9,000 ppm) was the only toxin detected in the rice medium on which this strain was grown. Rice cultured with this isolate was fed to 27 rats (3 rats per treatment) in a 1:1 and 1:9 ratio with complete rat diet respectively. All rats died within 16 hr and post mortem examination showed hemorrhage of the small intestines (Table 1). Each extract of rice cultured with MT-6 was intubated into rats at a level of 2.5 g and 1.25 g equivalents of rice culture dissolved in 0.5 ml 20% ethanol. Extraction with polar solvents (95% acetonitrile in water, MeOH and 50% aq. MeOH) of the rice caused the same deleterious signs in rats while the chloroform and ethylacetate extracts were not toxic. The red pigment(s) obtained by acetone extraction when administered by intubation into rats at a level of 25 g-equivalents of rice culture per 0.5 ml 20% ethanol, produced no toxic effects in the rats (Table 2). The residues of rice medium, left after extraction with polar solvents, were fed in a 1:1 mixture with complete rat diet and caused hemorrhaging of the small intestines and death. Repeated polar solvent extractions (6 to 7 times) of the cultured rice removed the toxic substance(s) as indicated by a lack of clinical signs when the residue was fed to rats (Table 1).

Incorporation of 8,000, 5,000, and 3,000 ppm (μg/g) purified (99%) moniliformin in complete rat diet caused hemorrhaging in the small intestine and death in 3 out of 3 rats within 16 hr (Table 3). Levels of 2,500 and 2,000 ppm moniliformin diet caused intestinal hemorrhaging and death in 2 out of 3 rats within 16 hr. Rats receiving a diet containing moniliformin at a concentration of 1,000 ppm (μg/g) and higher resulted in death of rats. Diets containing levels of 750, 500, and 250 ppm of moniliformin were not lethal and produced no visible lesions.

Intubation of purified moniliformin caused hemorrhage of the small intestine and death within 16 hr in 5 out of 5 rats at levels of 100, 80, 60, and 40 mg/kg body weight. Four of 5 rats intubated with 20 mg moniliformin/kg body weight also died within 16 hr. No toxic effects were observed in rats receiving moniliformin at levels of 10, 5, and 2.5 mg/kg within 16 hr (Table 4). Twenty-day-old-female rats either intubated with 10 mg/kg pure moniliformin or receiving <1,000 ppm of moniliformin in rat diet [from culture or standard] were without toxic effect within the first 24 hr. However, purified moniliformin intubated into 20-day-old-female rats at 20 mg/kg or fed a diet containing ⩾1,500 ppm caused toxic effects within the first 24 hr of the experiment. Similar toxic effects have been observed in other species (ducklings, mice, sheep and rats) receiving doses of moniliformin (Allen *et al.* 1981; Burmeister *et al.* 1979, 1980; Kriek *et al.* 1977; Lamprecht *et al.* 1986).

Rats are able to tolerate small quantities (<1,000 ppm) of

moniliformin in their diet. However, larger amounts were lethal whether given by intubation (>1.0 mg/kg) body weight as the pure toxin or as the dietary crude extract (>1,000 ppm) of rice medium. The rat feeding experiments performed indicate moniliformin whether pure or contained in crude extract from MT-6 grown on rice in culture is responsible for the toxicity observed. The residue from extracts in which moniliformin was not detected caused no observable toxicity.

This study has assisted us in the evaluation of hundreds of toxic isolates of *Fusarium* grown on a rice medium. In evaluation, it is important to determine whether the known toxins found account for the toxicity. This is of particular significance when searching for toxins which have not been characterized as yet. Moniliformin is produced by approximately 50% of the isolates tested and its presence might mask unknown toxins. This study indicates that moniliformin found in culture at a concentration of 1,000 ppm (1,000 μg/g) or higher and when incorporated into a rat diet, most likely accounts for the toxicity observed.

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